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Patent application No.: PA 1998 01102

Date of filing: 02 September 1998

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Title: Vision-based isolation of islets of Langerhans.

IPC: -

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Patent- og Varemærkestyrelsen
Økonomi- og Erhvervsministeriet

08 January 2004

John Nielsen

PATENT- OG VAREMÆRKESTYRELSEN

I, the undersigned Merete Ek, BUDDE, SCHOU & OSTENFELD A/S, Vester Søgade 10, DK-1601 Copenhagen V, Denmark, do hereby declare that I am proficient in both the English and Danish languages, and I certify the following to be a true and faithful English translation of the Danish-language patent specification DK PA 1998 01102 filed on 2 September 1998.

Witness under my hand

Copenhagen, 8 January 2004

Merete Ek

Søren Gregersen Confidential material 30.08.1998

1 September 1998

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Vision-based isolation of islets of Langerhans

Søren Gregersen Confidential material 30.08.1998

Title: Vision-based isolation of islets of Langerhans: with the possibility of computer control, documentation and automation.

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Background.

Diabetes is characterised by a defect in the insulin-producing β-cells in the pancreas. The β-cells are arranged in the so-called islets of Langerhans in the exocrine tissue. The ability of the β-cells to respond differentiatedly to concentration of blood glucose is essential to the maintenance of a normal metabolism in the body. The accessibility of isolated islets from animals and humans plays an essential role in connection with 1. the exploration of physiologic and patophysiologic mechanisms in the endocrine pancreas and 2. for animal as well as for human transplantations. The transplantation of islets of Langerhans include a potential treatment of diabetes. Furthermore, there is a great need of isolated islets for screening and testing of insulinotrope, new potential antidiabetica. Consequently, there is a strong need of automated methods for a fast and secure 'large-scale' isolation of islets.

Actual methods:

The actual techniques for isolation of islets of Langerhans often include injection of collagenase into the duct system of the pancreas. Hereby pancreas is disintegrated and after rinsing of the tissue the islets may be isolated. Subsequently, the islets may be isolated in several ways, e.g. by manual 'picking' under a stereo microscope; the islets are being sucked up into a special glass pipette or they are being transferred to a 'spoon'. Both methods require identification by means of vision. Worldwide many laboratories make use of manual isolation because this is the most gentle method for the islets.

During recent years methods for rinsing up islets on gradients (Ficoll/Percoll) which are advantageously applied to e.g. fetal islets of rats have been described. A number of other methods have been described in the literature. For isolation of islets from *dogs, pigs or humans* gradient centrifuging is mostly used. The present methods have a number of *disadvantages* , which in brief may be described as follows: 1. manual islet-isolation is extremely time-consuming, 2. there is an inter-operator variation, 3. no fast transfer of the islets to food medium is secured, 4. the islets may be damaged by centrifuging and by osmotic gradients.

OBJECT OF THE INVENTION

To provide an apparatus for automatic isolation of islets of Langerhans which apparatus at the same time makes it possible to describe the physical characteristics and selectors of the islets (e.g. based on size and shape).

CONCEPT

The apparatus is by means of digitised image handling (*Digital Imaging, Image Analysis)* to localise the islets of Langerhans in the tissue suspension and to isolate said islets. Further, digital imaging makes it possible to describe the physical data of the islets and to automate the process of islet isolation.

Advantages of Digital Imaging assisted islet isolation.

A number of demands are set up in order to secure the physical and functional integrity of the islets optimally as compared to the regular methods for islet isolation: 1. the islets must in no way be damaged, neither physically nor chemically, 2. the islets must in every respect act as islets isolated by means of traditional methods (as far as appearance, secretion pattern etc. are concerned). 3. the apparatus must be able to recognise and isolate the islets without using dying- or antisubstances, 4. the apparatus works at 4^0 C, and 5. the risk of contamination is reduced since the pipettes, tubes etc. may be autoclaved or are made of disposable material.

Solution to the problem I and II

Both the solutions to the problem are based on photo detection of the islets (digital imaging). Dependant on the solution to the problem(I and/or II) the islets are isolated by means of:

I. Detection and separation of the islets in a thin glass tube (or channel).
II. Sucking up of islets with a pipette which is moved around in a Petri dish (or any other plane surface containing the tissue suspension) by computer-controlled movements.

'Claims' comprise:
Identification (visual and/or digitised) of the biologic material as mentioned below in

I. (Solution to the problem I): A capillary tube, or in a duct, carried out in transparent (clear) material, the inner diameter being slightly bigger than the biggest islets or cell clusters.
II. (Solution to the problem II): A Petri dish, or any other plane surface containing the tissue suspension.

The term 'the method' is meant to include the below solution to the problem I or II.

As to the biological material, said material comprising:
1. The use of the method for isolation of the islets of Langerhans from any species, including humans. The islets of Langerhans may be surrounded by any possible material, biological or non-biological (e.g. for preventing an immunity reaction in connection with transplantation). The islets/cell clusters* may be marked with antisubstance or other substance added for the purpose of identification and/or analysis.
2. The use of the method for describing physical/chemical/biochemical characteristics of the islets of Langerhans.
3. The use of the method for isolation or description of physical/chemical/biochemical characteristics of the exocrine tissue.
4. The use of the method for isolating or describing physical/chemical/biochemical characteristics of other cell clusters than the islets of Langerhans.

5. The use of the method for any subsequent cleaning out procedure or selection of islets than those mentioned under point 1. I.e. it also includes a possible use of the method for isolation or selection of islets which primarily (i.e. the first isolation procedure after removal of pancreas) may be isolated according to another method.

Re cell cluster*
The term 'cell cluster' is meant to include naturally or artificially originated multitudes of cells of 3-100.000 cells from any vertebrate and/or ex-corpori originated cell multitudes, e.g. cancer cells. Cell clusters originated from cell lines. Also genetically engineered islets or cell clusters are included. Multitudes of cells comprising originally monocellular organisms are also included.
Antisubstance-, or in any other way conjugated cells resulting in multitudes of cells are included.

TECHNICAL DESCRIPTION OF THE INVENTION

Vision-based identification of, and subsequently, isolation of islets of Langerhans. More specifically the identification takes place in either:

I A capillary tube, or in a duct, carried out in transparent (clear) material, which as far as the islet-isolation is concerned, has an inner diameter of 0.25-3 µm and a length of app. 5-35 mm. As to other cell clusters being slightly bigger than the diameter of the biggest clusters. The inner shape of the tube may be of any possible geometrical cross-sectional shape, e.g. circular or quadrangular, and the length may be e.g. conical.
II A Petri dish, or any other plane surface containing the tissue suspension.

The visual identification may be performed by all wavelengths and in case an antisubstance or other substances have been added to the tissue to the islets/cell clusters for the purpose of identification and/or analysis there might also be a possibility of transmitting the light of one wavelength to the tissue and let the camera detect light of another wavelength. It might be a possibility to insert filters in the light source or in the camera.

Solution to the problem I (vide Fig. I)

The principle in this model is that a camera provided with a zoom lens (A) scans a capillary tube or duct (C). The camera is connected to a computer with a program for digital image analysis (B). The capillary tube or duct is through a thin tube (E) connected to a reservoir (F) with the tissue suspension. Said reservoir is constantly and slowly (app. 10 rpm) rotating by means of a controlled electric engine (G) in order to keep the tissue in homogeneous suspension.

Either by applying a pressure to the reservoir or by means of the gravitation, the tissue suspension is allowed to pass through the capillary tube or duct (C) at a carefully matched speed permitting the identification in the capillary tube to be performed. The flow speed being calculated on basis of the movement of the particles in the capillary tube, the period of time before the micro pump (H) is to be activated is calculated. The micro pump delivers a short-term fluid flow (perpendicular, or almost perpendicular, to the capillary tube) resulting in the islet/cell cluster changing its direction and moving out into a side tube (I) through a tube leading into a flask (J) with cultivation medium. The islets may also be transferred directly to other mediums (e.g. to incubation vials in connection with tests and/or analysis work).

If convenient, the micro pump (H) may be substituted by an electromagnetically controlled valve which provides 'guiding' the islet out into the side tube (I).

The tube or channel/duct in which the identification is made is illuminated by a light source (not shown in Fig. I). Vide comments on page 4.

Solution to problem II (vide Fig. II)

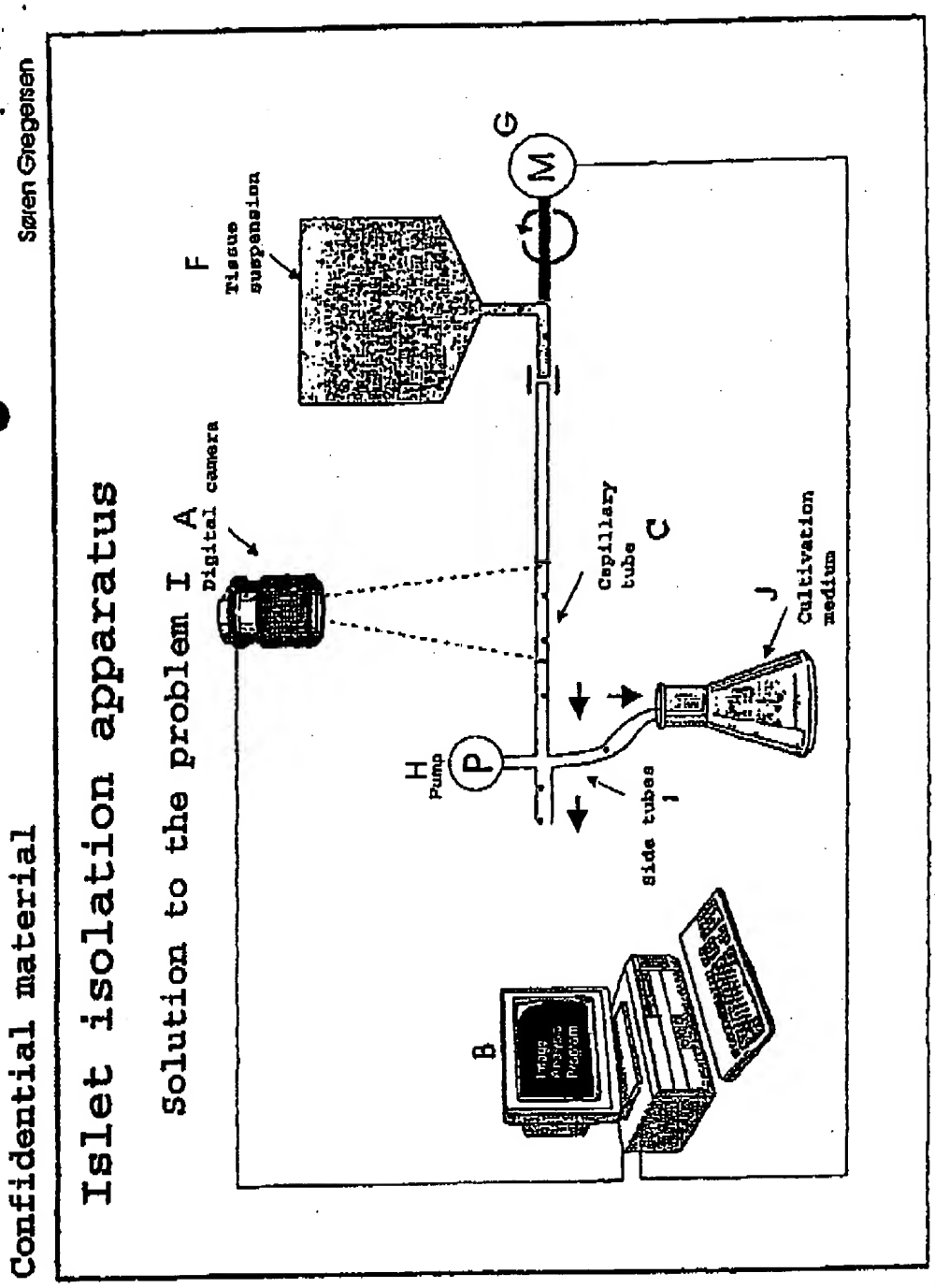
A camera (A) attached to a computer with a program for digital imaging analysis (B) scans a Petri dish (C) or any other plane surface containing tissue suspension. The camera and the pipette (D) (which may be constructed to form the same unit) are controlled on the x-y-z level by computer-controlled electric engines (E). The point of

the pipette is visible in the frame section of the camera. The pipette is linked to a plastics tube (F) which leads into a flask (G) containing medium, the islets/cell clusters being transferred to said flask. The islets/cell clusters may also be transferred directly to other mediums (e.g. to incubation vials in connection with tests and/or analysis work). By way of a suction (H) the flask (G) has a small partial vacuum resulting in the islets being sucked through the pipette and tube. A computer-controlled valve (I) controlling the suction in the pipette (D) is placed on the tube (F).

The control of the z-y-x levels (E) is carried out with a degree of accuracy being sufficient to secure the suction of one single islet (i.e. an accuracy on all levels of app. 0,05 mm).

The tissue suspension is illuminated by a light source (not shown in Fig. I). Vide comments on page 4.

Århus, / 1998 Søren Gregersen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Islet isolation apparatus
Solution to the problem I
A
Digital camera
F
Tissue suspension
G
M
Capillary tube
C
J
Cultivation medium
H
Pump
P
side tubes
I
B

Confidential material

Islet isolation apparatus

Solution to the problem II

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